ATGGCAAATA AAGCAGTAAA TGACTTTATA CTAGCTATGA 40

ATTACGATAA AAAGAAACTC TTGACCCATC AGGGAGAAAG 80

TATTGAAAAT CGTTTCATCA AAGAGGGTAA TCAGCTACCC 120

GATGAGTTTG TTGTTATCGA AAGAAAGAAG CGGAGCTTGT 160

CGACAAATAC AAGTGATATT TCTGTAACAG CTACCAACGA 200

CAGTCGCCTC TATCCTGGAG CACTTCTCGT AGTGGATGAG 240

ACCTTGTTAG AGAATAATCC CACTCTTCTT GCGGTCGATC 280

GTGCTCCGAT GACTTATAGT ATTGATTTGC CTGGTTTGGC 320

AAGTAGCGAT AGCTTTCTCC AAGTGGAAGA TCCCAGCAAT 360

TCAAGTGTTC GCGGAGCGGT AAACGATTTG TTGGCTAAGT 400

GGCATCAAGA TTATGGTCAG GTCAATAATG TCCCAGCTAG 440

AATGCAGTAT GAAAAAATCA CGGCTCACAG CATGGAACAA 480

CTCAAGGTCA AGTTTGGTTC TGACTTTGAA AAGACAGGGA 520

ATTCTCTTGA TATTGATTTT AACTCTGTCC ATTCAGGCGA 560

AAAGCAGATT CAGATTGTTA ATTTTAAGCA GATTTATTAT 600

ACAGTCAGCG TAGACGCTGT TAAAAATCCA GGAGATGTGT 640

TTCAAGATAC TGTAACGGTA GAGGATTTAA AACAGAGAGG 680

AATTTCTGCA GAGCGTCCTT TGGTCTATAT TTCGAGTGTT 720

GCTTATGGGC GCCAAGTCTA TCTCAAGTTG GAAACCACGA 760

GTAAGAGTGA TGAAGTAGAG GCTGCTTTTG AAGCTTTGAT 800

AAAAGGAGTC AAGGTAGCTC CTCAGACAGA GTGGAAGCAG 840

ATTTTGGACA ATACAGAAGT GAAGGCGGTT ATTTTAGGGG 880

GCGACCCAAG TTCGGGTGCC CGAGTTGTAA CAGGCAAGGT 920

GGATATGGTA GAGGACTTGA TTCAAGAAGG CAGTCGCTTT 960

FIG. 1

EXPRESS MAIL NO.
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ACAGCAGATC ATCCAGGCTT GCCGATTTCC TATACAACTT 1000
CTTTTTTACG TGACAATGTA GTTGCGACCT TTCAAAATAG 1040
TACAGACTAT GTTGAGACTA AGGTTACAGC TTACAGAAAC 1080
GGAGATTTAC TGCTGGATCA TAGTGGTGCC TATGTTGCCC 1120
AATATTATAT TACTTGGAAT GAATTATCCT ATGATCATCA 1160
AGGTAAGGAA GTCTTGACTC CTAAGGCTTG GGACAGAAAT 1200
GGGCAGGATT TAACGGCTCA CTTTACCACT AGTATTCCTT 1240
TAAAAGGGAA TGTTCGTAAT CTCTCTGTCA AAATTAGAGA 1280
GTGTACCGGG CTTGCTTGGG AATGGTGGCG TACGGTTTAT 1320
GAAAAAACCG ATTTGCCACT AGTGCGTAAG CGGACGATTT 1360
CTATTTGGGG AACAACTCTC TATCCGCAGG TAGAAGATAA 1400
GGTAGAAAAT GAC 1413

FIG. 1

ATGGCAAATA AAGCAGTAAA TGACTTTATA CTAGCTATGA 40

ATTACGATAN$_{50}$ AAAN$_{54}$AAACTC TTGACCCATC AGGGAGAAAG 80

TATTGAAAAT CGTTTCAN$_{98}$CA AAGAGGGTAA TCAGCTACCC 120

GN$_{122}$TGAGTTTG TTGN$_{134}$TAN$_{137}$CGA AAGAAAGAAG CGGAGCTTGT 160

CGACAAATAC AAGTGATATT N$_{181}$CTGTAN$_{187}$CAG CTACCN$_{196}$ACGA 200

CAGTCGCCTC TATCCTGGAG CACTTCTCGT AGTGGATGAG 240

ACCTTGTN$_{248}$AG AGAATAATCC CACTCTTCTT GCGGTN$_{276}$GATC 280

GTGCTCCGAT GACTTATAGT AN$_{302}$TGN$_{305}$TTTGC CTGGTTTGGC 320

AAGTAGCGAT AGCTTTCTCC AAGTGGAAGA N$_{351}$CCCAGCAAT 360

TCAAGTGTTC GCGGAGCGGN$_{380}$ AN$_{382}$ACGATTTG TTGGCTAAGT 400

GGCATCAAGA TTATGGTCAG GTCAATAATG TCCCAGCTAG 440

AAN$_{443}$GCAGTAT GAAAAAATN$_{459}$A CGGCTCACAG CATGGAACAA 480

CTCAAGGTCA AGTTTGGTTC TGACTTTGAA AAGN$_{514}$CAGGGA 520

ATTCTCTTGA TATTGATTTT AACTCTGTCC ATTCAGGN$_{558}$GA 560

AAAGCN$_{566}$GATT CAGATTGTTA ATN$_{583}$TTAAGCA GATTTATTAT 600

ACAGTCAGCG TAGACGCTGT TAAAAATCCA GGAGATGTGT 640

TTCAAGATAC TGTAACGGTA GAGGATTTAA AACAGAGAGG 680

AATTTCTGCA GAGCGTCCTT TGGTCTATAT TTCGAGN$_{717}$GTT 720

GCTTATGGGC GCCAAGTCTA TCTCAAGTTG GAAACCACGA 760

GTAN$_{764}$GAGTGN$_{770}$ TGAAGTAGAG GCTGCTTTTG AAGCTTTGAT 800

AAAAGGAGTC AAGGTAGCTC CTCAGACAGA GTGGAAGCAG 840

ATTTTGGACA ATACAGAAGT GAAGGCGGTT ATTTTAGGGG 880

GCGACCCAAG TTCGGGTGCC CGAGTTGTAA CAGGCAAGGT 920

GGATATGGTA GAGGACTTGA TTCAAGAAGG CAGTCGCTTT 960

ACAGCAGATC ATCCAGGCTT GCCGATTTCC TATACAACTT 1000

FIG. 2

CTTTTTTACG TGACAATGTA GTTGCGACCT TTCAAAAN$_{1038}$AG 1040

TACAGACTAT GTTGAGACTA AGGTTACAGC TTACAGAAAC 1080

GGAGATTTAC TGCTGGATCA TAGTGGTGCC TATGTTGCCC 1120

AATATTATAT TACTTGGN$_{1138}$AT GAATTATCCT ATGATCATCA 1160

AGGTAAGGAA GTCTTGACTC CTAAGGCTTG GGACAGAAAT 1200

GGGCAGGATT TN$_{1212}$ACGGCTCA CTTTACCACT AGTATTCCTT 1240

TAAAAGGGAA TGTTCGTAAT CTCTCTGTCA AAATTAGAGA 1280

GTGTACCGGG CTTGCN$_{1296}$TGGG AATGGTGGCG TACGGTTTAT 1320

GAAAAAACCG ATTTGCCACT AGTGCGTAAG CGGACGATTT 1360

CTATTTGGGG AACAACTCTC TATCCN$_{1386}$CAGG TAGAN$_{1395}$GATAA 1400

GGTAGAAAAT GAC 1413

FIG. 2

```
Met Ala Asn Lys Ala Val Asn Asp Phe Ile Leu Ala
1               5                  10
Met Asn Tyr Asp Lys Lys Lys Leu Leu Thr His Gln
            15                  20
Gly Glu Ser Ile Glu Asn Arg Phe Ile Lys Glu Gly
 25                  30                  35
Asn Gln Leu Pro Asp Glu Phe Val Val Ile Glu Arg
                40                  45
Lys Lys Arg Ser Leu Ser Thr Asn Thr Ser Asp Ile
     50                  55                  60
Ser Val Thr Ala Thr Asn Asp Ser Arg Leu Tyr Pro
                65                  70
Gly Ala Leu Leu Val Val Asp Glu Thr Leu Leu Glu
            75                  80
Asn Asn Pro Thr Leu Leu Ala Val Asp Arg Ala Pro
 85                  90                  95
Met Thr Tyr Ser Ile Asp Leu Pro Gly Leu Ala Ser
            100                 105
Ser Asp Ser Phe Leu Gln Val Glu Asp Pro Ser Asn
    110                 115                 120
Ser Ser Val Arg Gly Ala Val Asn Asp Leu Leu Ala
                125                 130
Lys Trp His Gln Asp Tyr Gly Gln Val Asn Asn Val
            135                 140
Pro Ala Arg Met Gln Tyr Glu Lys Ile Thr Ala His
145                 150                 155
Ser Met Glu Gln Leu Lys Val Lys Phe Gly Ser Asp
                160                 165
Phe Glu Lys Thr Gly Asn Ser Leu Asp Ile Asp Phe
    170                 175                 180
Asn Ser Val His Ser Gly Glu Lys Gln Ile Gln Ile
                185                 190
Val Asn Phe Lys Gln Ile Tyr Tyr Thr Val Ser Val
            195                 200
Asp Ala Val Lys Asn Pro Gly Asp Val Phe Gln Asp
205                 210                 215
Thr Val Thr Val Glu Asp Leu Lys Gln Arg Gly Ile
                220                 225
Ser Ala Glu Arg Pro Leu Val Tyr Ile Ser Ser Val
    230                 235                 240
Ala Tyr Gly Arg Gln Val Tyr Leu Lys Leu Glu Thr
                245                 250
Thr Ser Lys Ser Asp Glu Val Glu Ala Ala Phe Glu
            255                 260
Ala Leu Ile Lys Gly Val Lys Val Ala Pro Gln Thr
265                 270                 275
Glu Trp Lys Gln Ile Leu Asp Asn Thr Glu Val Lys
                280                 285
```

FIG. 3

```
Ala Val Ile Leu Gly Gly Asp Pro Ser Ser Gly Ala
    290                 295                 300
Arg Val Val Thr Gly Lys Val Asp Met Val Glu Asp
                305                 310
Leu Ile Gln Glu Gly Ser Arg Phe Thr Ala Asp His
        315                 320
Pro Gly Leu Pro Ile Ser Tyr Thr Thr Ser Phe Leu
325                 330                 335
Arg Asp Asn Val Val Ala Thr Phe Gln Asn Ser Thr
            340                 345
Asp Tyr Val Glu Thr Lys Val Thr Ala Tyr Arg Asn
    350                 355                 360
Gly Asp Leu Leu Leu Asp His Ser Gly Ala Tyr Val
                365                 370
Ala Gln Tyr Tyr Ile Thr Trp Asn Glu Leu Ser Tyr
        375                 380
Asp His Gln Gly Lys Glu Val Leu Thr Pro Lys Ala
385                 390                 395
Trp Asp Arg Asn Gly Gln Asp Leu Thr Ala His Phe
            400                 405
Thr Thr Ser Ile Pro Leu Lys Gly Asn Val Arg Asn
    410                 415                 420
Leu Ser Val Lys Ile Arg Glu Cys Thr Gly Leu Ala
                425                 430
Trp Glu Trp Trp Arg Thr Val Tyr Glu Lys Thr Asp
        435                 440
Leu Pro Leu Val Arg Lys Arg Thr Ile Ser Ile Trp
445                 450                 455
Gly Thr Thr Leu Tyr Pro Gln Val Glu Asp Lys Val
            460                 465
Glu Asn Asp
    470
```

FIG. 3

Met Ala Asn Lys Ala Val Asn Asp Phe Ile Leu Ala
1 5 10
Met Asn Tyr Asp Xaa Xaa Lys Leu Leu Thr His Gln
15 20
Gly Glu Ser Ile Glu Asn Arg Phe Xaa Lys Glu Gly
25 30 35
Asn Gln Leu Pro Xaa Glu Phe Val Xaa Xaa Glu Arg
40 45
Lys Lys Arg Ser Leu Ser Thr Asn Thr Ser Asp Ile
50 55 60
Xaa Val Xaa Ala Thr Xaa Asp Ser Arg Leu Tyr Pro
65 70
Gly Ala Leu Leu Val Val Asp Glu Thr Xaa Leu Glu
75 80
Asn Asn Pro Thr Leu Leu Ala Val Asp Arg Ala Pro
85 90 95
Met Thr Tyr Ser Xaa Xaa Leu Pro Gly Leu Ala Ser
100 105
Ser Asp Ser Phe Leu Gln Val Glu Asp Pro Ser Asn
110 115 120
Ser Ser Val Arg Gly Ala Xaa Xaa Asp Leu Leu Ala
125 130
Lys Trp His Gln Asp Tyr Gly Gln Val Asn Asn Val
135 140
Pro Ala Arg Xaa Gln Tyr Glu Lys Xaa Thr Ala His
145 150 155
Ser Met Glu Gln Leu Lys Val Lys Phe Gly Ser Asp
160 165
Phe Glu Lys Xaa Gly Asn Ser Leu Asp Ile Asp Phe
170 175 180
Asn Ser Val His Ser Gly Glu Lys Xaa Ile Gln Ile
185 190
Val Asn Xaa Lys Gln Ile Tyr Tyr Thr Val Ser Val
195 200
Asp Ala Val Lys Asn Pro Gly Asp Val Phe Gln Asp
205 210 215
Thr Val Thr Val Glu Asp Leu Lys Gln Arg Gly Ile
220 225
Ser Ala Glu Arg Pro Leu Val Tyr Ile Ser Xaa Val
230 235 240
Ala Tyr Xaa Arg Gln Val Tyr Leu Lys Leu Glu Thr
245 250
Thr Ser Xaa Ser Xaa Glu Val Glu Ala Ala Phe Glu
255 260
Ala Leu Ile Lys Gly Val Lys Val Ala Pro Gln Thr
265 270 275
Glu Trp Lys Gln Ile Leu Asp Asn Thr Xaa Val Lys
280 285

FIG. 4

```
Ala Val Ile Leu Gly Gly Asp Pro Ser Ser Gly Ala
    290                 295                 300
Arg Val Val Thr Gly Lys Val Asp Met Val Glu Asp
                305                 310
Leu Ile Gln Glu Gly Ser Arg Phe Thr Ala Asp His
        315                 320
Pro Gly Leu Pro Ile Ser Tyr Thr Thr Ser Phe Leu
325                 330                 335
Arg Asp Asn Val Val Ala Thr Phe Gln Asn Ser Thr
            340                 345
Asp Tyr Val Glu Thr Lys Val Thr Ala Tyr Arg Asn
    350                 355                 360
Gly Asp Leu Leu Leu Asp His Ser Gly Ala Tyr Val
                365                 370
Ala Gln Tyr Tyr Ile Thr Trp Xaa Glu Leu Ser Tyr
        375                 380
Asp His Gln Gly Lys Glu Val Leu Thr Pro Lys Ala
385                 390                 395
Trp Asp Arg Asn Gly Gln Asp Leu Thr Ala His Phe
            400                 405
Thr Thr Ser Ile Pro Leu Lys Gly Asn Val Arg Asn
    410                 415                 420
Leu Ser Val Lys Ile Arg Glu Cys Thr Gly Leu Ala
                425                 430
Trp Glu Trp Trp Arg Thr Val Tyr Glu Lys Thr Asp
        435                 440
Leu Xaa Leu Val Arg Lys Arg Thr Ile Ser Ile Trp
445                 450                 455
Gly Thr Thr Leu Tyr Pro Gln Val Glu Asp Lys Val
            460                 465
Glu Asn Asp
    470
```

FIG. 4

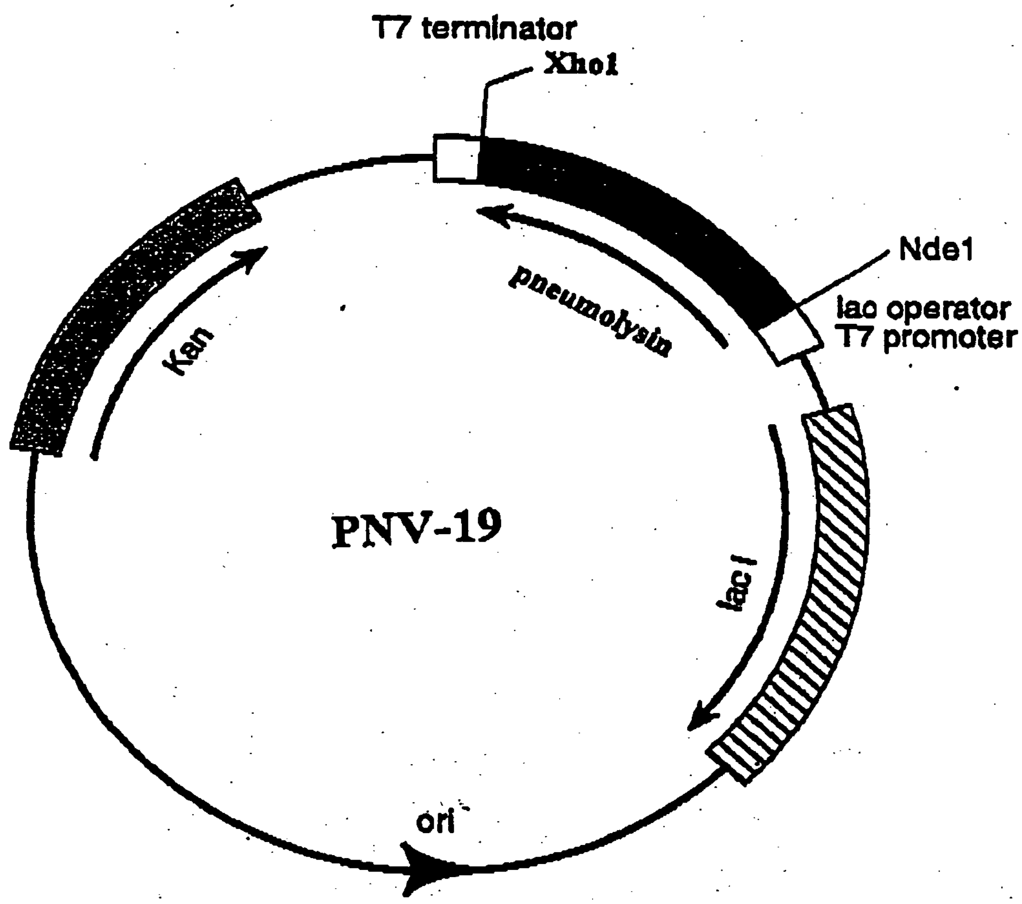
T7 terminator
XhoI
NdeI
lac operator
T7 promoter
pneumolysin
Kan
PNV-19
lac I
ori

Fig. 5

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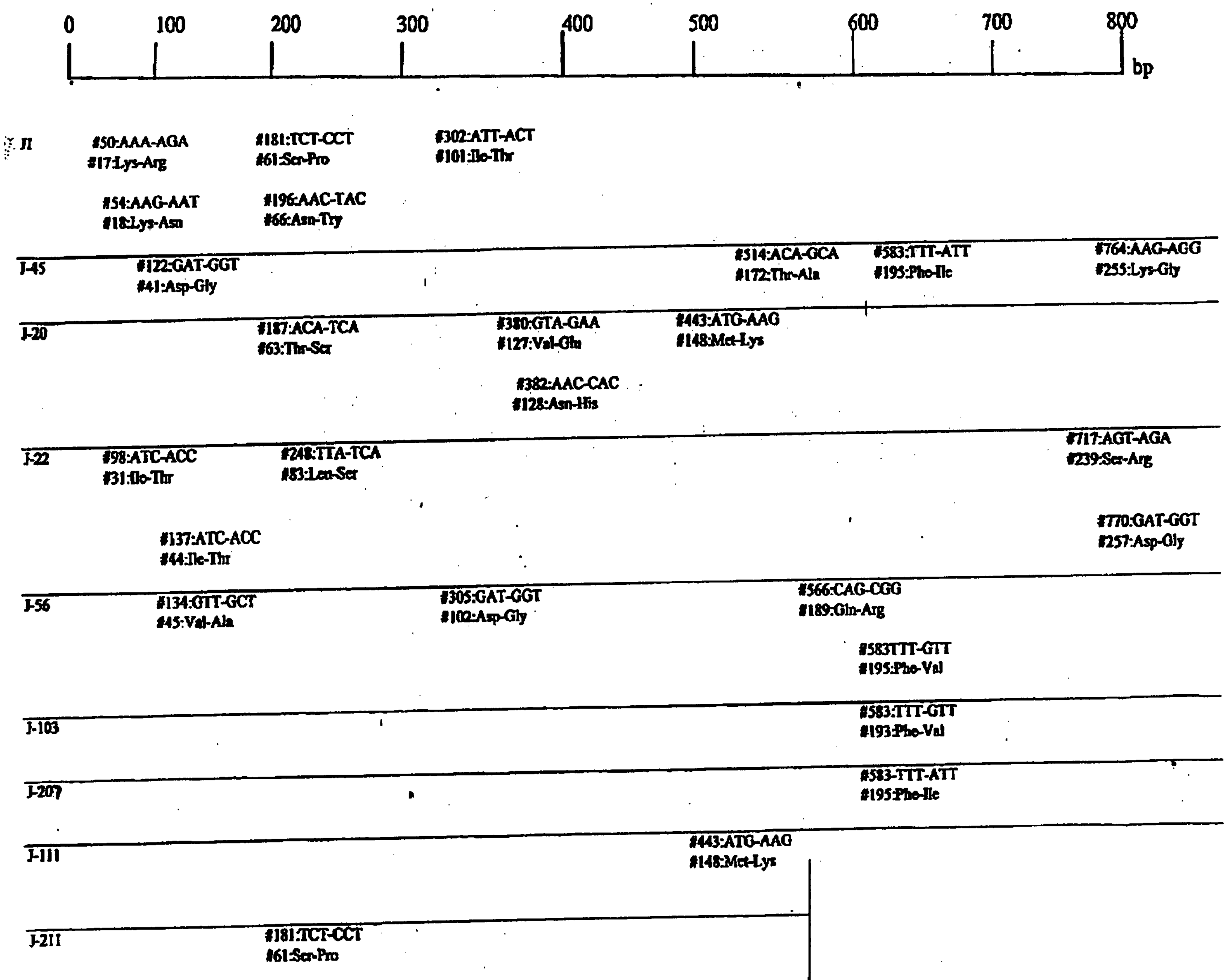


Fig. 6

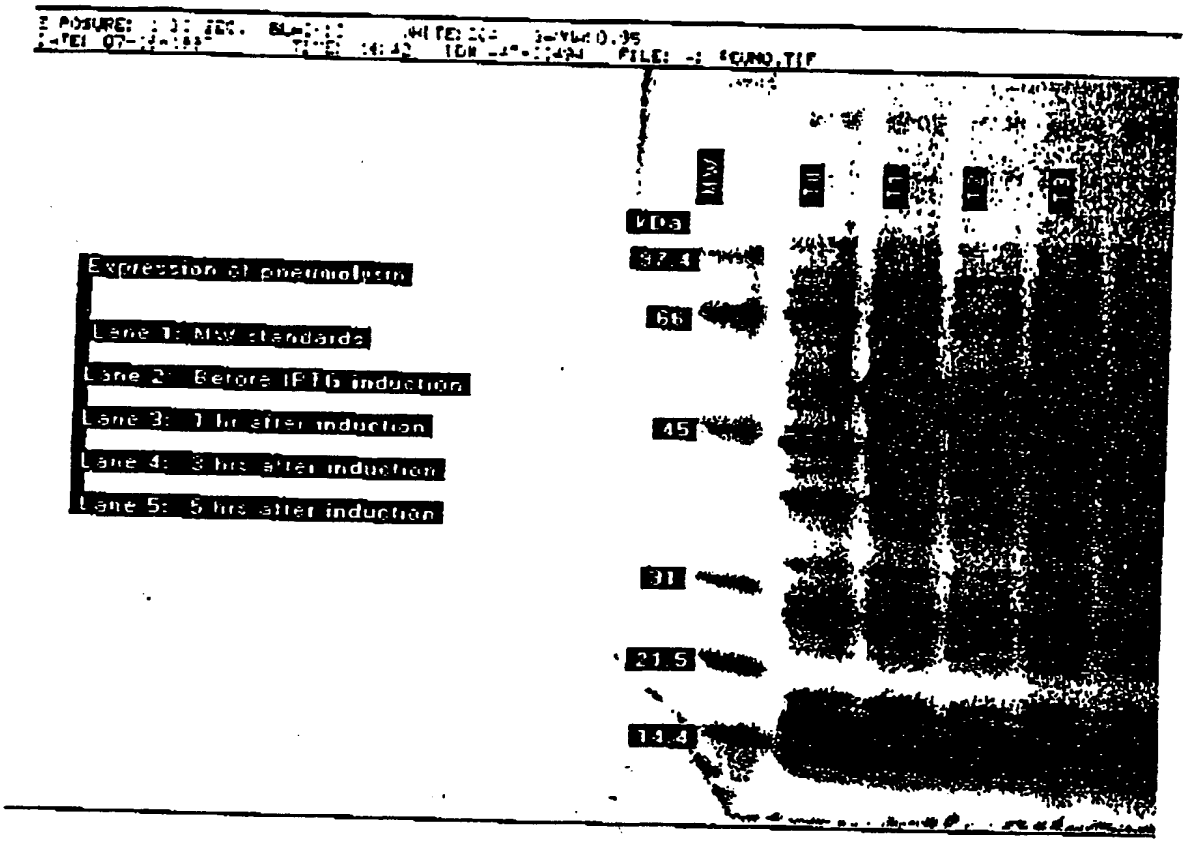
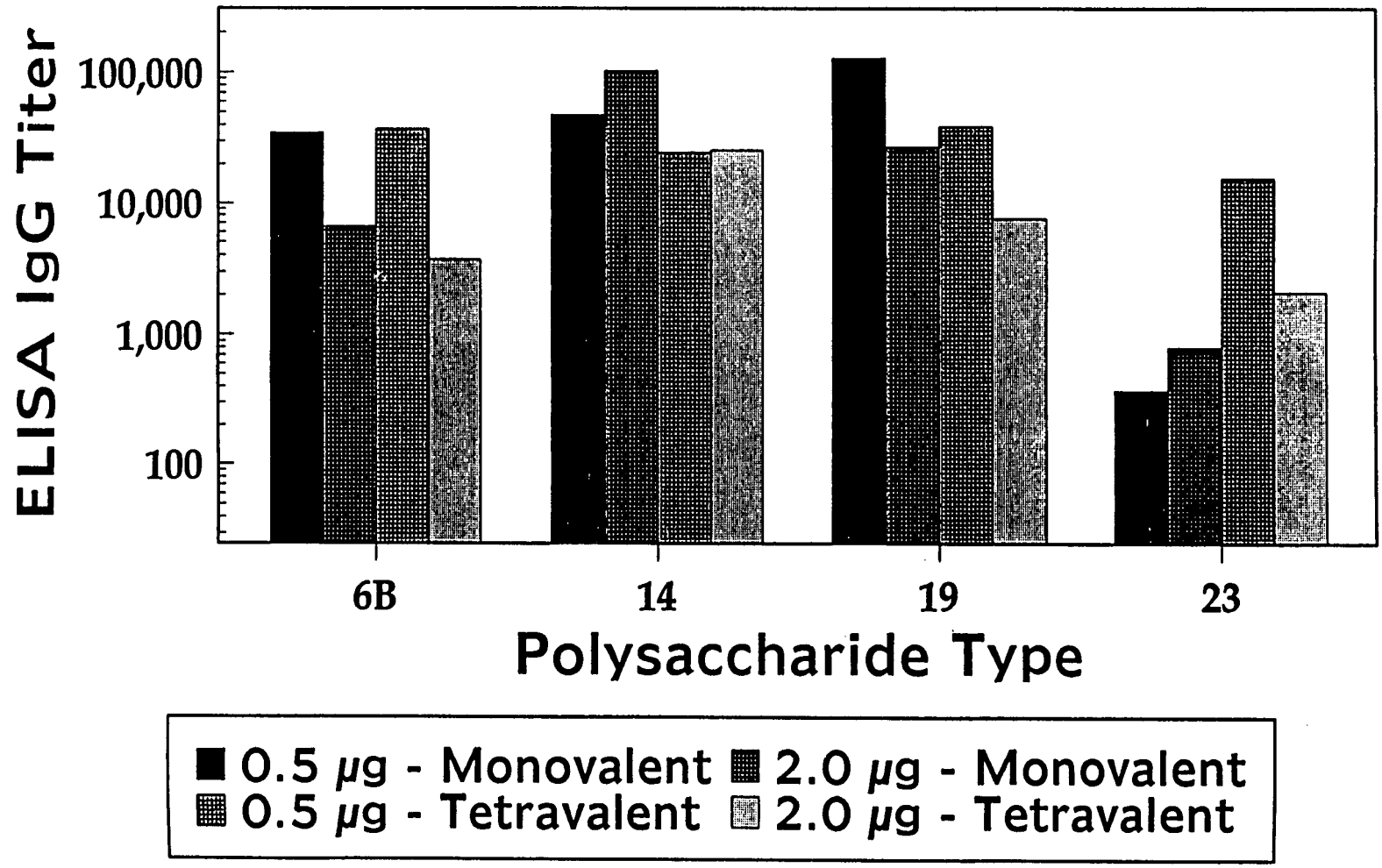

Expression of pneumolysin
Lane 1: MW standards
Lane 2: Before IPTG induction
Lane 3: 1 hr after induction
Lane 4: 3 hrs after induction
Lane 5: 5 hrs after induction
MW
kDa
97.4
66
45
31
21.5
14.4

Fig. 7

FIG. 8

Polysaccharide Dose Response for Pneumococcal Pneumolysin Conjugates in Mice after 2 Injections: Polysaccharide-Specific IgG



Polysaccharide-Specific IgG for Tetravalent Pneumococcal Conjugates in Mice after 2 Injections: Pneumolysin versus Tetanus Toxoid Carriers

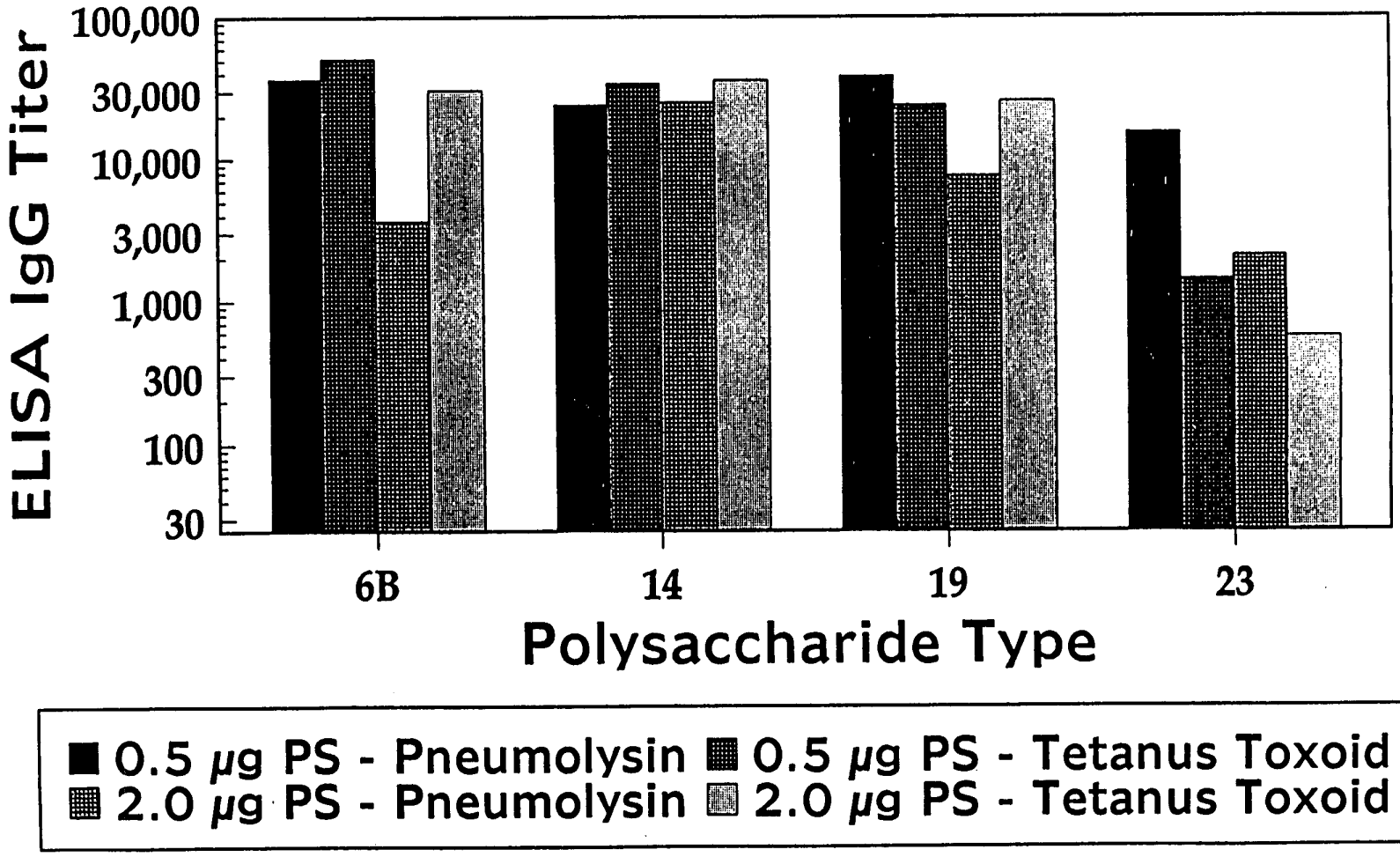


FIG 9

Pneumolysin-Specific IgG Elicited by Monovalent & Tetravalent Pneumococcal Polysaccharide-Pneumolysin Conjugates in Mice after 2 Injections

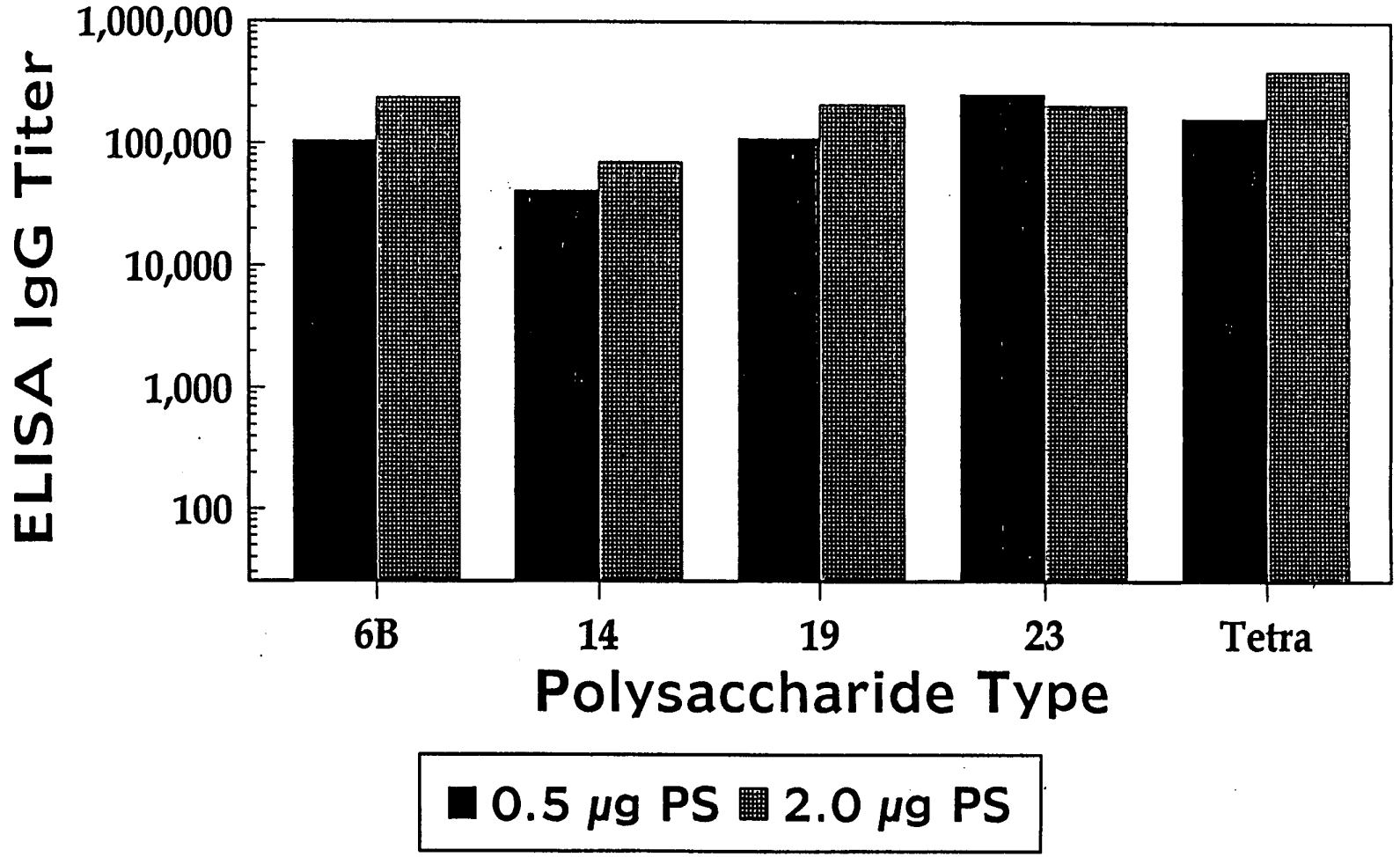


FIG. 10

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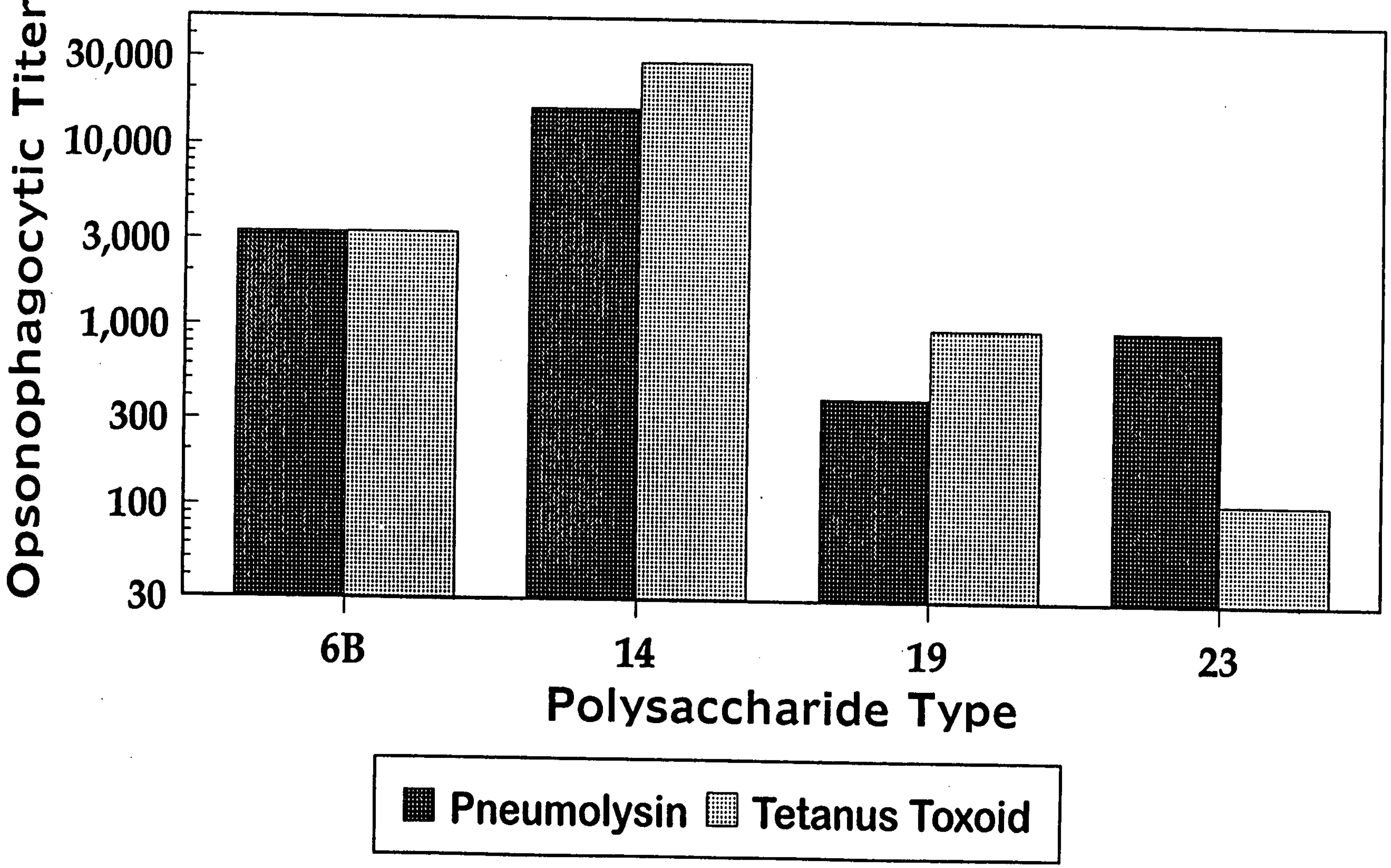
FIG. 11

Polysaccharide (PS)-Specific Opsonophagocytic Activity Elicited by Tetravalent Pneumococcal PS-Pneumolysin and PS-Tetanus Toxoid Conjugate Vaccines in Mice after 2 Injections



Anti-Hemolytic Pneumolysin-Specific Activity Elicited by Monovalent & Tetravalent Pneumococcal Conjugates in Mice after 3 Injections

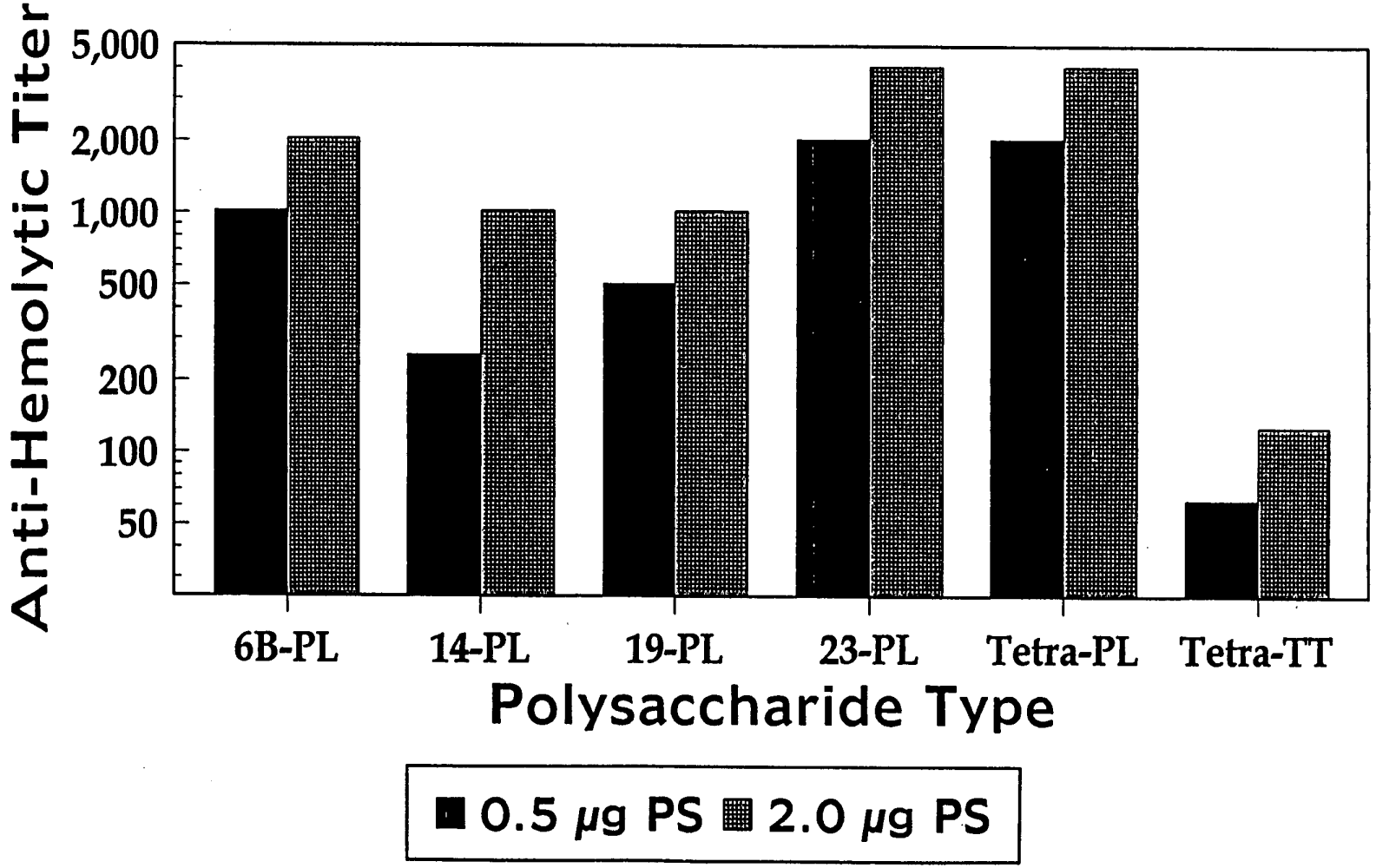


FIG. 12

EXPRESS MAIL NO.
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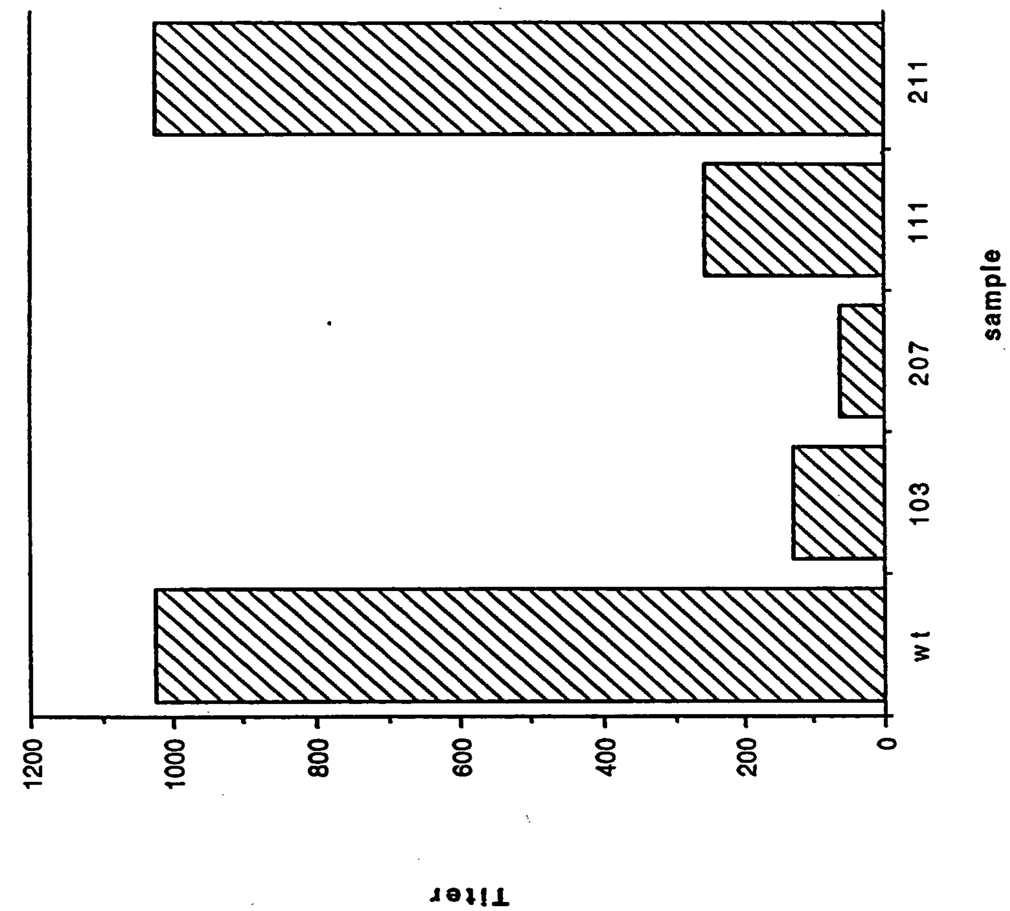

FIG 13

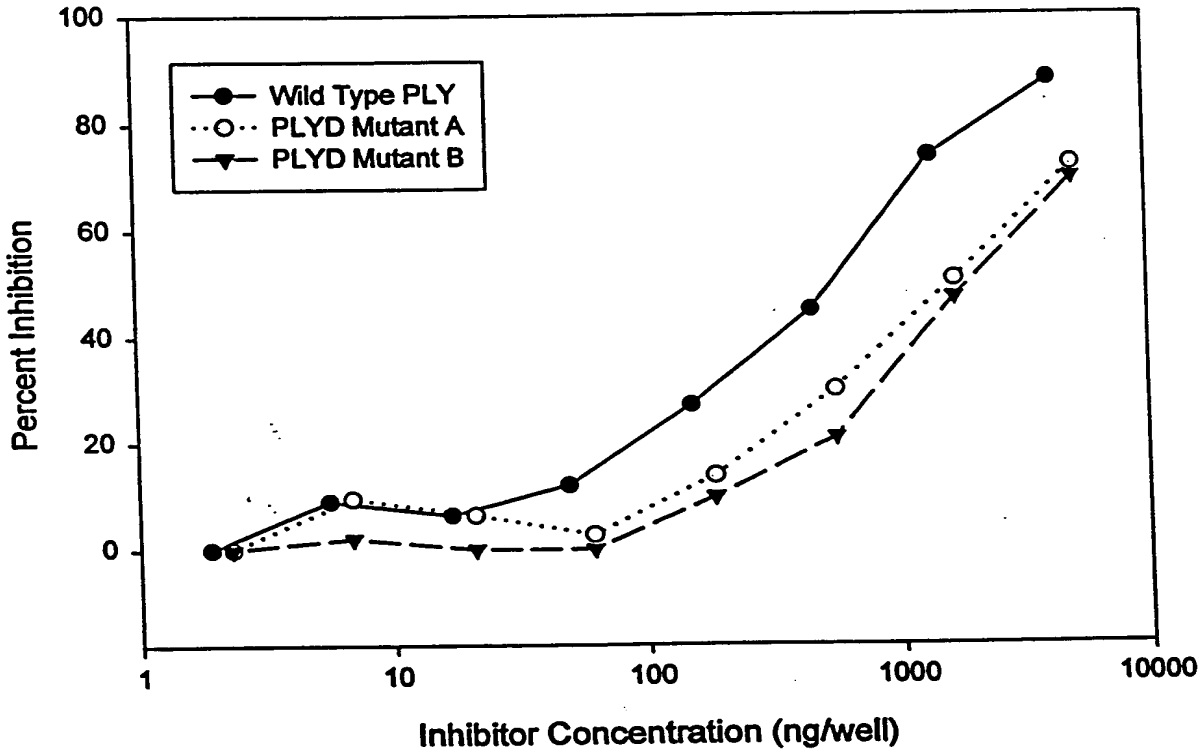

Fig 14.

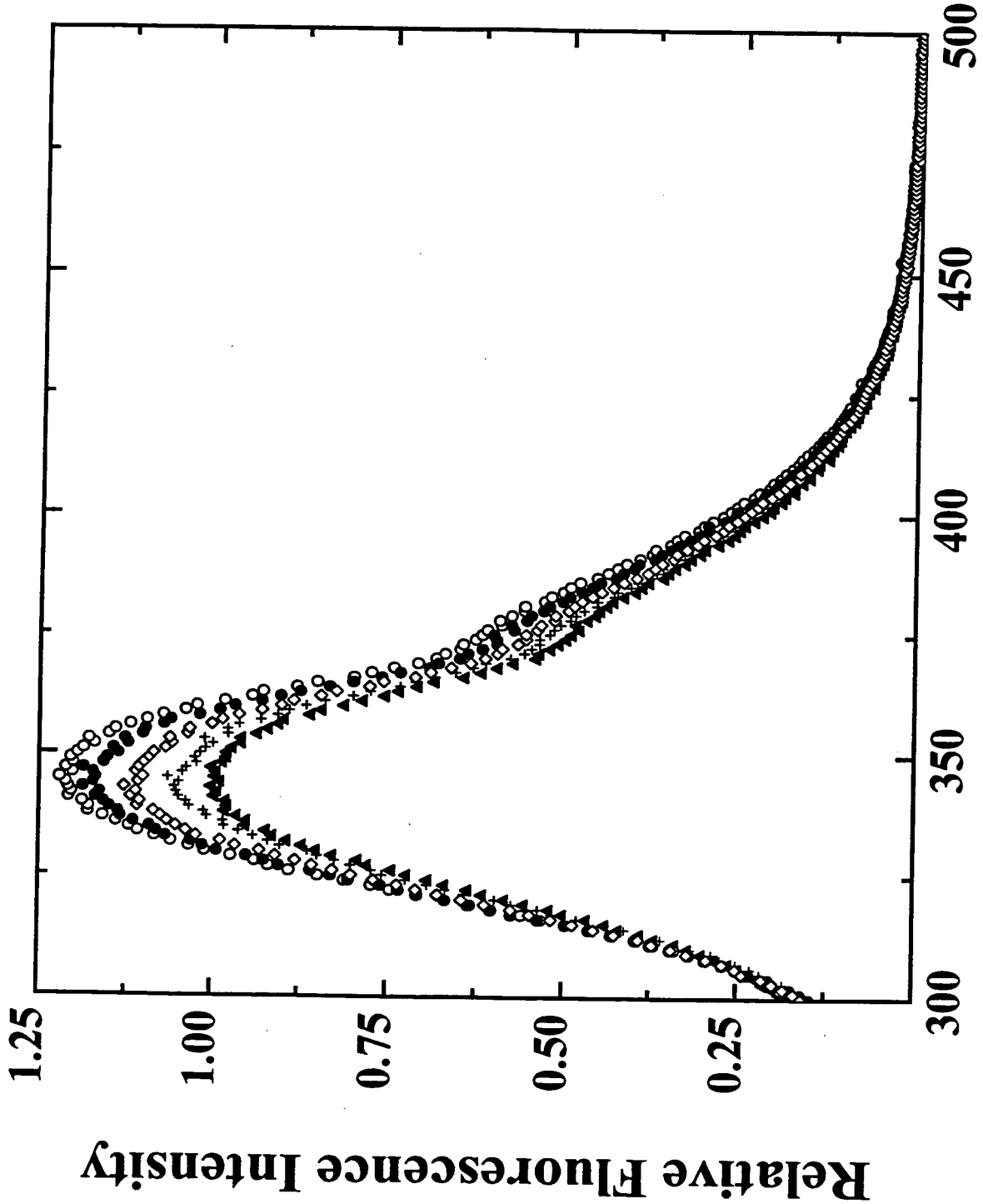

FIG 15

CD Spectra of Mutant Pneumolysin (Free Protein and Conjugate)

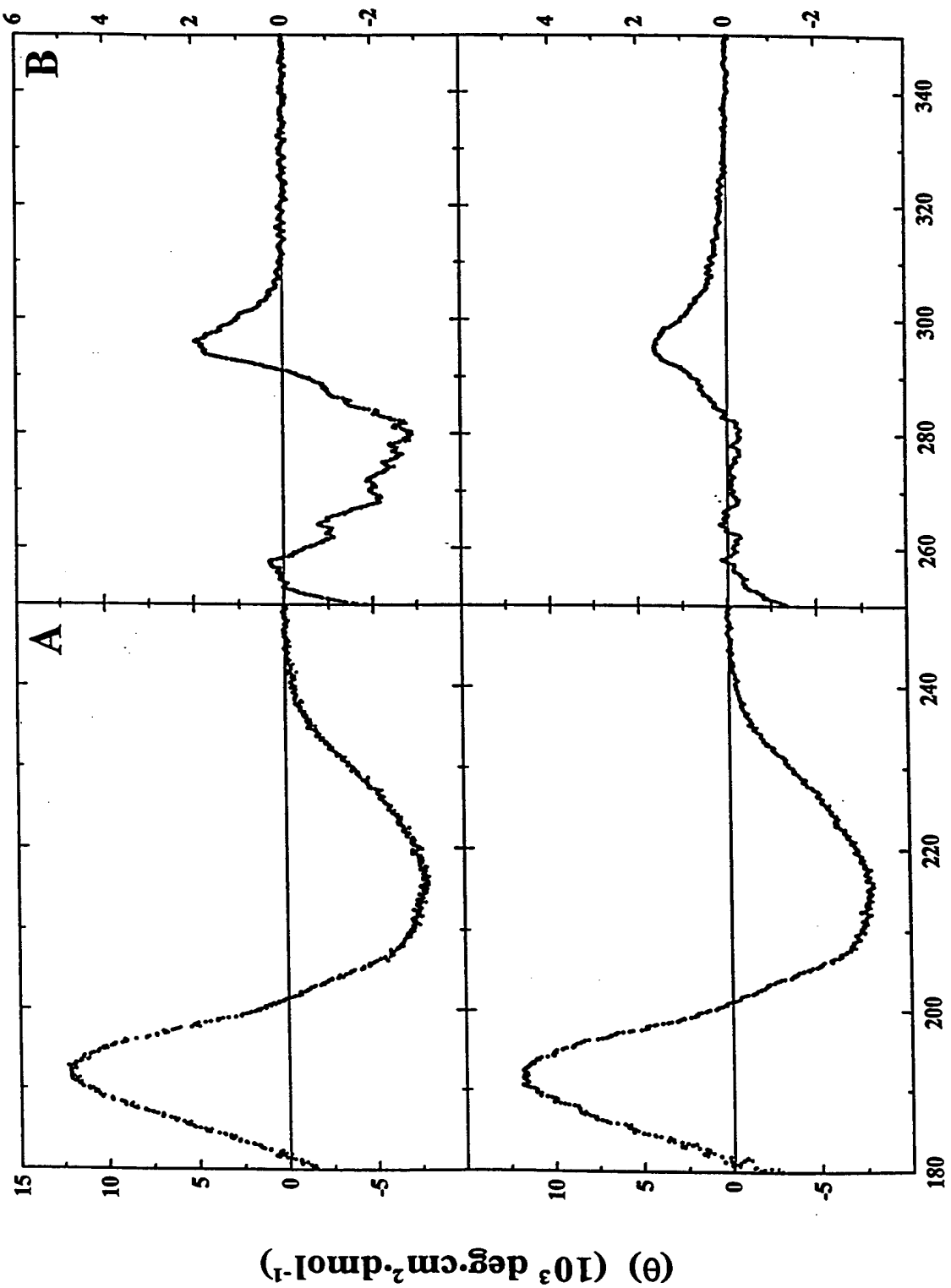

FIG 16

Tetravalent Pneumococcal Pneumolysoid Conjugate Vaccine in Mice: Polysaccharide-Specific IgG Response over Time

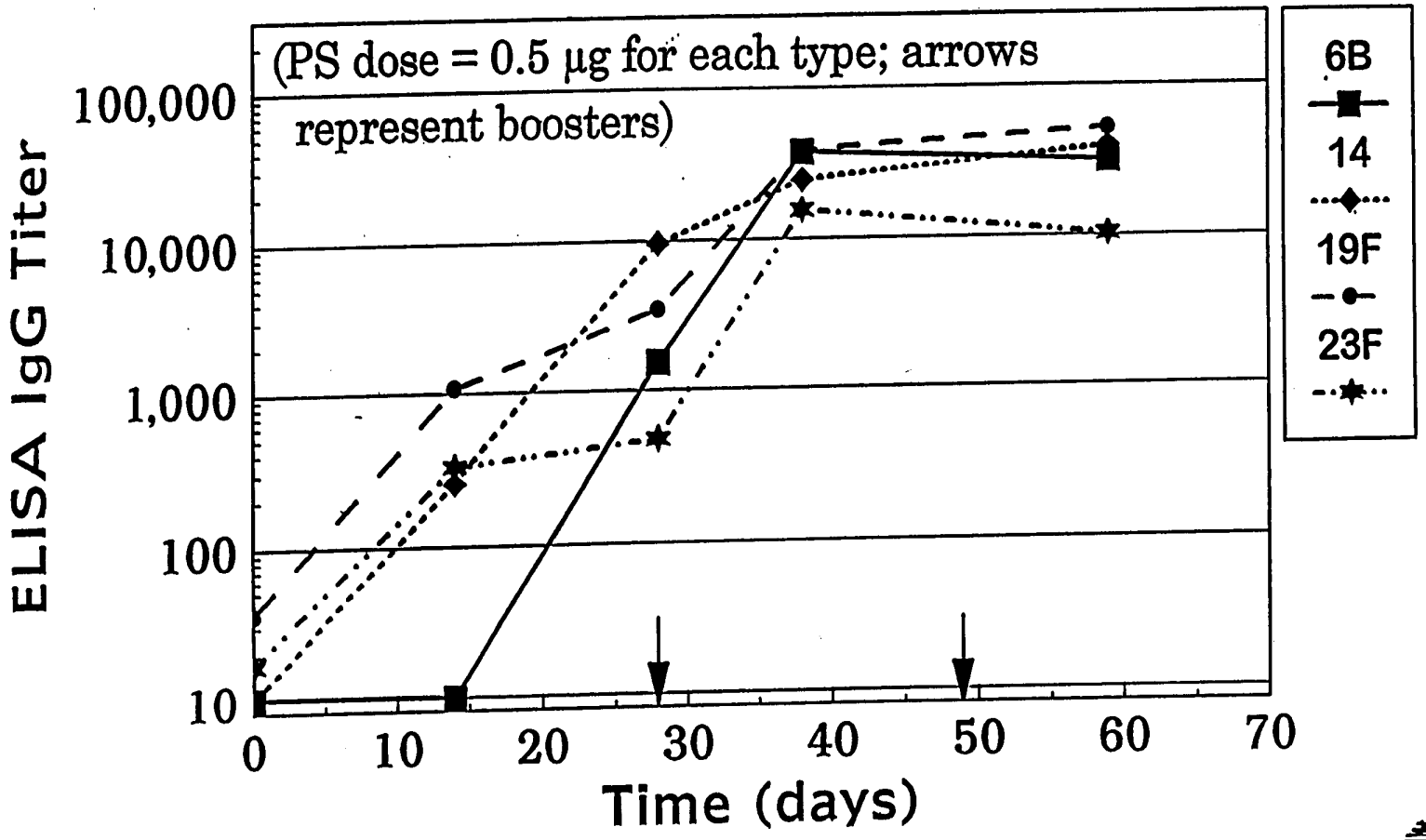

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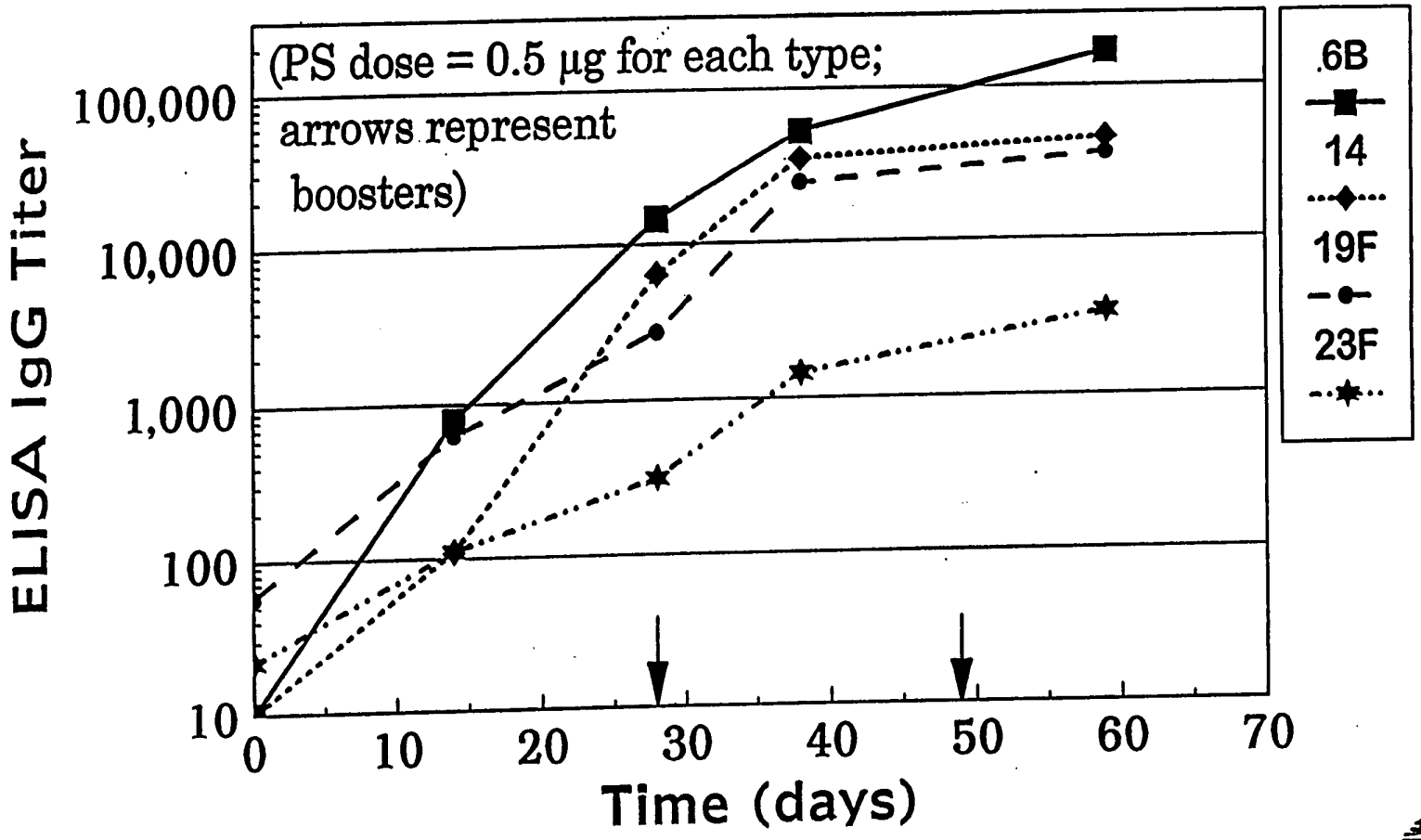
FIG 17A

Tetravalent Pneumococcal TT Conjugate Vaccine (2nd) in Mice: Polysaccharide-Specific IgG Response over Time



NORTH AMERICAN VACCINE, INC.

FIG 17B

EXPRESS MAIL NO.
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Monovalent Pneumococcal Pneumolysoid Conjugate Vaccines in Mice: Polysaccharide-Specific IgG Response over Time

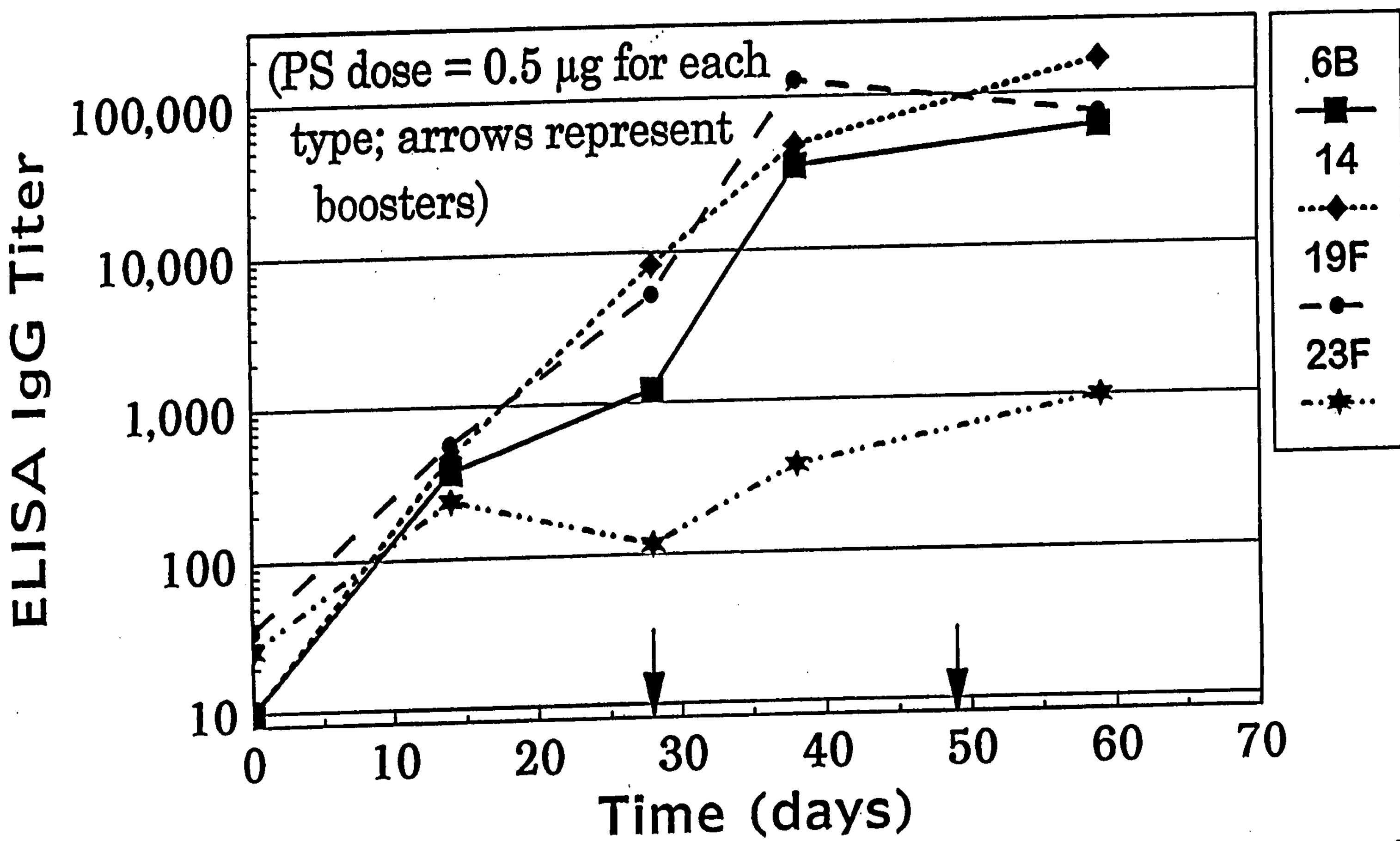

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FIG 17C